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The shell microstructure and chronology of the abalone *Haliotis corrugata*

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Abstract

The abalone *Haliotis corrugata* deposits fine and dark rings in the shell near the spire and the dark rings can be used to age the shell. Each dark ring has two distinct microstructural elements; a layer of prismatic structure, possibly calcite, and a juxtaposed narrower layer of granular structure. The crystalline ultrastructure of aragonite between the rings shows seasonal changes in thickness of laminae apparently correlated with seasonal temperature changes. Examination of shells grown in culture showed that three fine rings are deposited during the first year, the first dark ring at an age of about 20 months, and the second between 2 and 2 ½ years; thereafter a dark ring is deposited annually. This aging technique was validated up to about 12 years of age by using growth checks laid down in the shell in about October 1991 during the El Niño as a time-stamp from which growth rates and age could be estimated. Using the aging technique the von Bertalanffy growth equation was fitted to age-length data from La Natividad, Baja California and the following parameters obtained: K = 0.33; L_∞=160 mm. Catch curve analyses of commercial catch data gave Z = 0.28 for a sample in the late 1970s, and 0.66 for one in 1995.

Keywords: *Haliotis corrugata*; rings; shell-aging; shell ultrastructure; El Niño, growth check.

Introduction

The ability to age an abalone shell is valuable for fishery management because growth rates and mortality can be readily estimated without long and costly field experiments (Day & Fleming 1992). The abalone *Haliotis corrugata* Gray is a substantial component of the commercial abalone fishery in Mexico (Guzmán del Próo 1992), but its abundance has declined seriously in recent years possibly through overfishing. The biology of the species is poorly known, so there is a need for basic information on growth and mortality rates for better management of the fishery.

Following the discovery of rings in abalone shells by Hayashi (1955), Muñoz-Lopez (1976) first suggested that the shell of *H. corrugata* could be aged by counting the rings in the spire, but did not validate the technique. In recent studies of the shell of *H. fulgens*, Shepherd *et al.* (1995a) and Shepherd and Turrubiates (1997) have shown that prismatic layers (called rings), possibly calcitic (but see Hawkes *et al.* 1997) and deposited alternately with layers of aragonite in the spire, are a reliable indicator of age, although the picture is complicated by the presence of pigmented and non-pigmented rings. In this paper we examine the pattern of ring deposition in shells of *H. corrugata*. First, we describe salient features of the micro- and ultra-structure of the shell, and then examine the frequency of ring deposition in shells grown in culture. Lastly we apply the technique to samples of

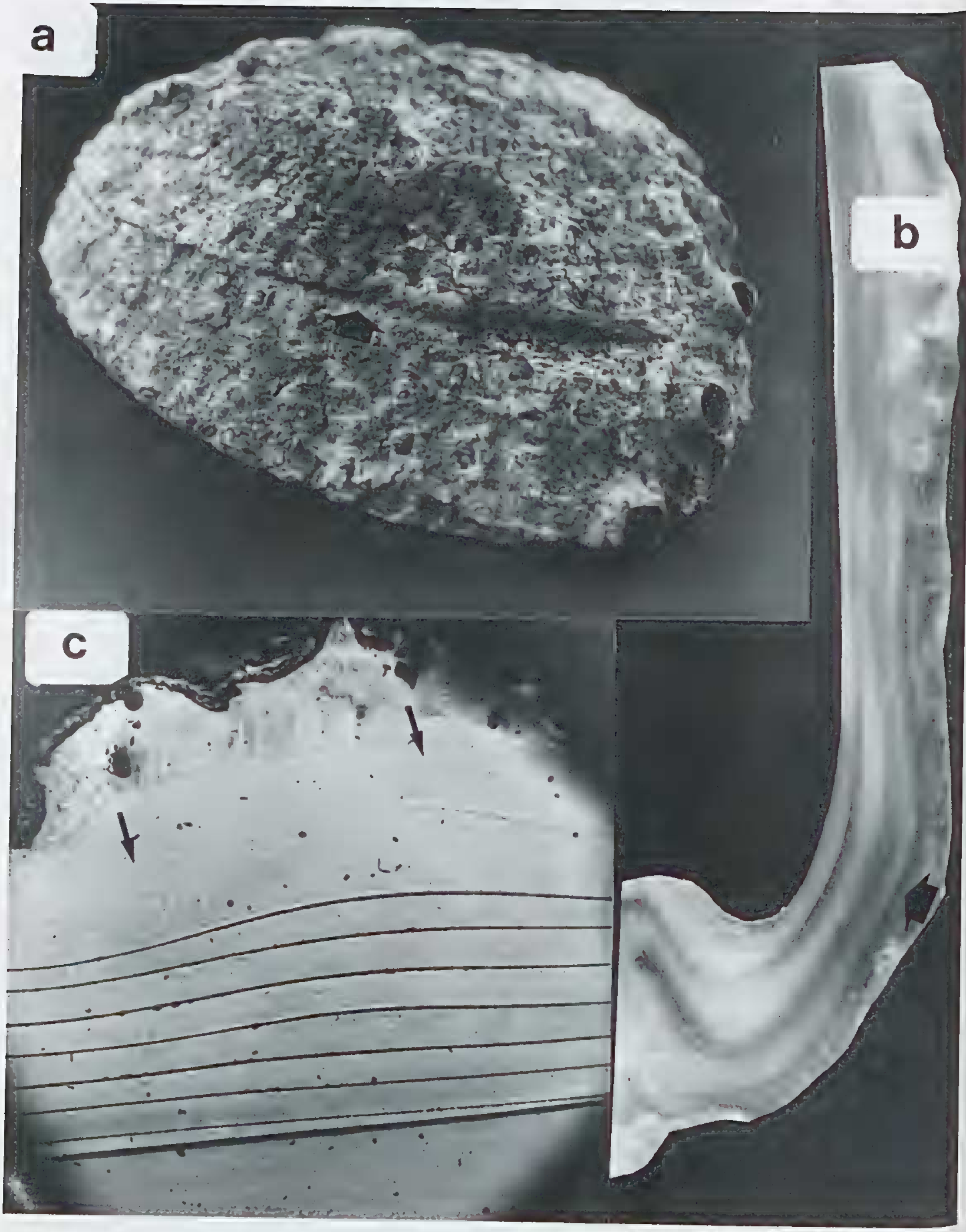

Figure1. a – photo of *H. corrugata* showing (arrowhead) prominent El Niño growth check; b – vertical section at the spire showing (arrowhead) the recommended area in which to count dark rings; c – micrograph of vertical section in the recommended area showing dark rings and (arrowheads) indistinct fine rings.

shells from La Natividad Island, Baja California (Lat.27°51'26"N; 115°07'30"W) and estimate growth and mortality rates.

The strong El Niño event of 1991–2 (Hayward *et al.* 1994) elevated sea temperatures some 2–3°C in central Baja California causing the disappearance of the kelp forests of *Macrocystis pyrifera* from about October 1991 until the end of the next year (J Turrubiates pers. comm.). El Niños can produce conspicuous growth checks in abalone shells (Cox 1962) and did so in 1991 in *H. fulgens* (Shepherd and Turrubiates 1997) and *H. corrugata*. So we used this event as a time stamp to validate the rate of deposition of rings in *H. corrugata*.

Materials and methods

A sample of 50 shells of *H. corrugata*, 12–71 mm shell length (SL) grown in culture in California (McCormick 1992) was examined. The mean growth rate of the shells was 14.3 mm per year (T. McCormick pers. comm.) and was linear with length over the size range examined, so the age of each shell was estimated from length. In addition we examined 224 shells from the commercial catch taken at La Natividad in March 1995, and 214 shells 60–177 mm SL taken during research surveys there from 1990–5.

The spires of the shells were ground with an electric grinder until a minute hole appeared, and then polished with 200 to 600 grit abrasives. These horizontal sections revealed a series of concentric rings. The number of rings was counted under a stereo microscope. Vertical sections of a sub-sample were cut at the spire, polished, set in resin, polished again with fine alumina powder, and etched with dilute HCl as described by Shepherd *et al.* (1995a). The variation in crystalline ultrastructure was examined across the inner nacreous layer of vertical sections by taking a series of micrographs (x5000) under the scanning electron microscope (SEM). The thickness of the laminae in the nacreous layers was then measured from the micrographs in a transect across the section from the outer to inner margin. Because of the difficulty of measuring individually laminae with fuzzy margins we measured groups of 10 and then calculated the mean width of individuals. In all 24 vertical sections were examined in detail, and the rings of 438 shells were examined in horizontal section.

Shells with "El Niño" growth checks (Fig.1a) were examined for growth in two ways. First, we measured shell length to the growth check (SL_1) and the total length (SL). Assuming (a) a uniform birth date on 1 November in accordance with current knowledge of peak spawning in *H. corrugata* (Ortiz *et al.* 1990), and (b) deposition of growth checks on 1 November 1991 (see results), we calculated and then plotted the mean annual increment $(SL-SL_1)/y$ vs the mean length, where y is the period in years from the El Niño date (1 Nov.1991) until the date of capture. This is the classical Gulland-Holt plot (Gulland 1983). A least squares regression analysis gives estimates of the growth parameters of the von Bertalanffy growth curve.

Next, we selected shells taken in March 1995 with El Niño growth checks such that $SL_1 < 140$ mm SL and estimated their age to SL_1 using mean age-length data derived from the growth analyses of the shells taken in March 1995. We added 3.3 years, the period from 1 November 1991 to March 1995, to give an estimated age of the shell, and compared the observed number of rings with the estimated age. The parameters of the von Bertalanffy growth equation were calculated for the age-length data by using a Ford-Walford plot (Gulland 1983) to facilitate comparison with earlier estimates of growth for this abalone. For the catch-curve analysis (Gulland 1983) we used the age-length data from commercial catch samples of 1994 and 1995 (N=221) to prepare an age-length key and applied that to length-frequency catch data from a midden believed to be of the late 1970s (N=228) on La Natividad and a sample (N=502) of March 1995.

Results

Microstructure

At low magnifications vertical sections of the shell of *H. corrugata* show clear growth rings near

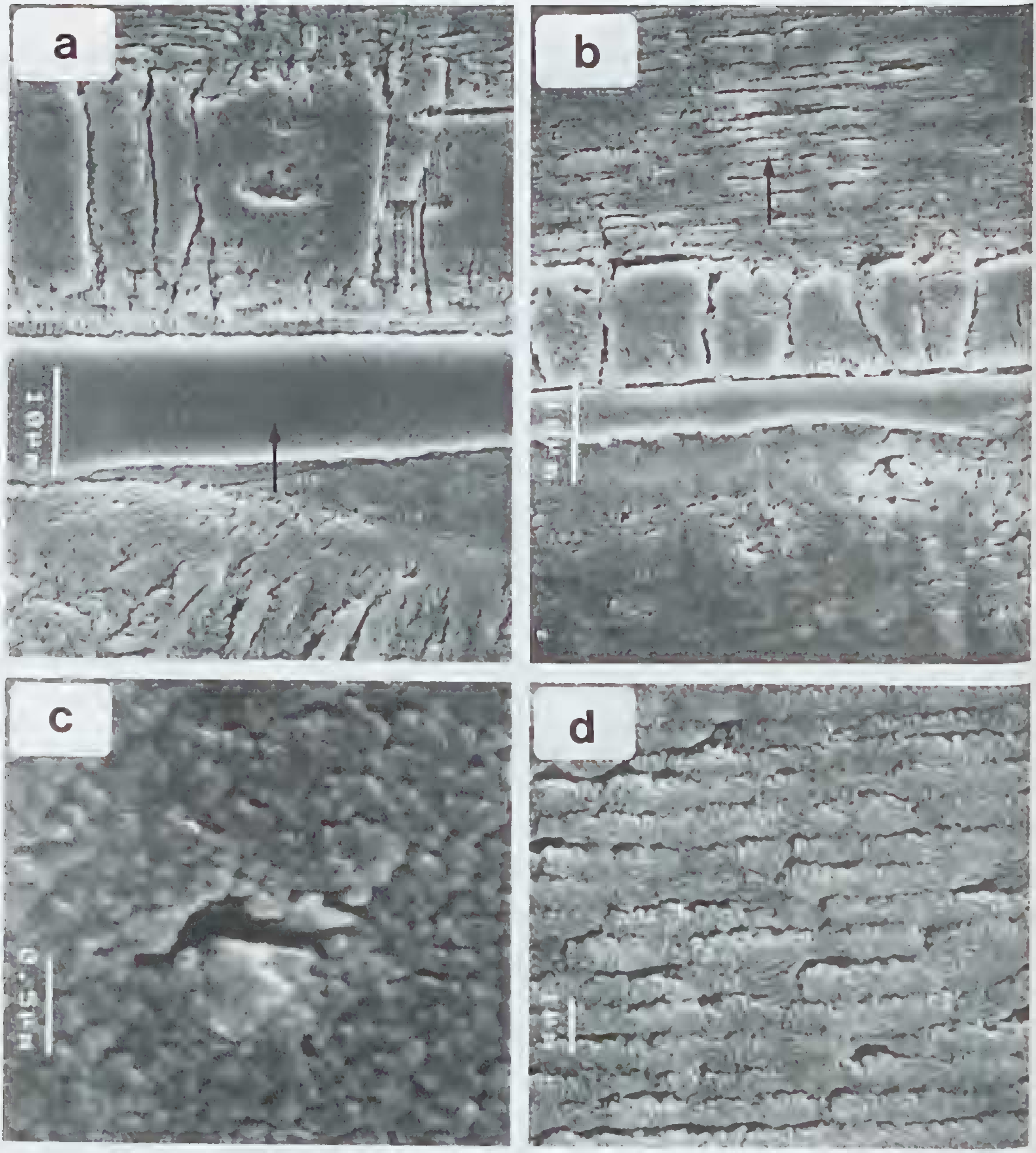

Figure 2. a – dark ring laid down at 8 years of age. The ring comprises block-like prisms above, and (arrow) layer of granular material below. b – dark ring laid down at 2–2 ½ years of age. Laminae of fairly uniform thickness (arrow) can be seen in the aragonitic nacreous layer laid down before the ring, whereas narrower, less regular laminae are laid down after the ring. Rings are laid down from top to bottom. c – micrograph of homogeneous granular material in lower part of ring. d – micrograph of laminae in aragonitic nacreous layer.

the spire (Fig. 1b,c). At higher magnification, up to three or four unpigmented rings (called fine rings) can be seen at the outer margin of the section close to the spire, but these disappear a short distance from it (Fig.1c). Pigmented rings (called dark rings) are then deposited sequentially toward the centre of the section and are conspicuous throughout the length of the section. SEMs show that the rings have a structure not previously described for abalone. Initially, block-like prisms are deposited on the aragonitic substratum (Fig.2a,b) and then a dark layer of homogeneous granular material is deposited (Fig.2a,b,c) giving way again to aragonitic laminae. In contrast to *H. fulgens* and *H. rubra*, the laminae are fuzzy at the edges (Fig.2d) due to the coarseness of the marginal granules.

The electron micrograph sequence shows a striking cyclic ultrastructural pattern of variation in laminar thickness between rings (Fig.3). A plot of the change in mean thickness along a transect

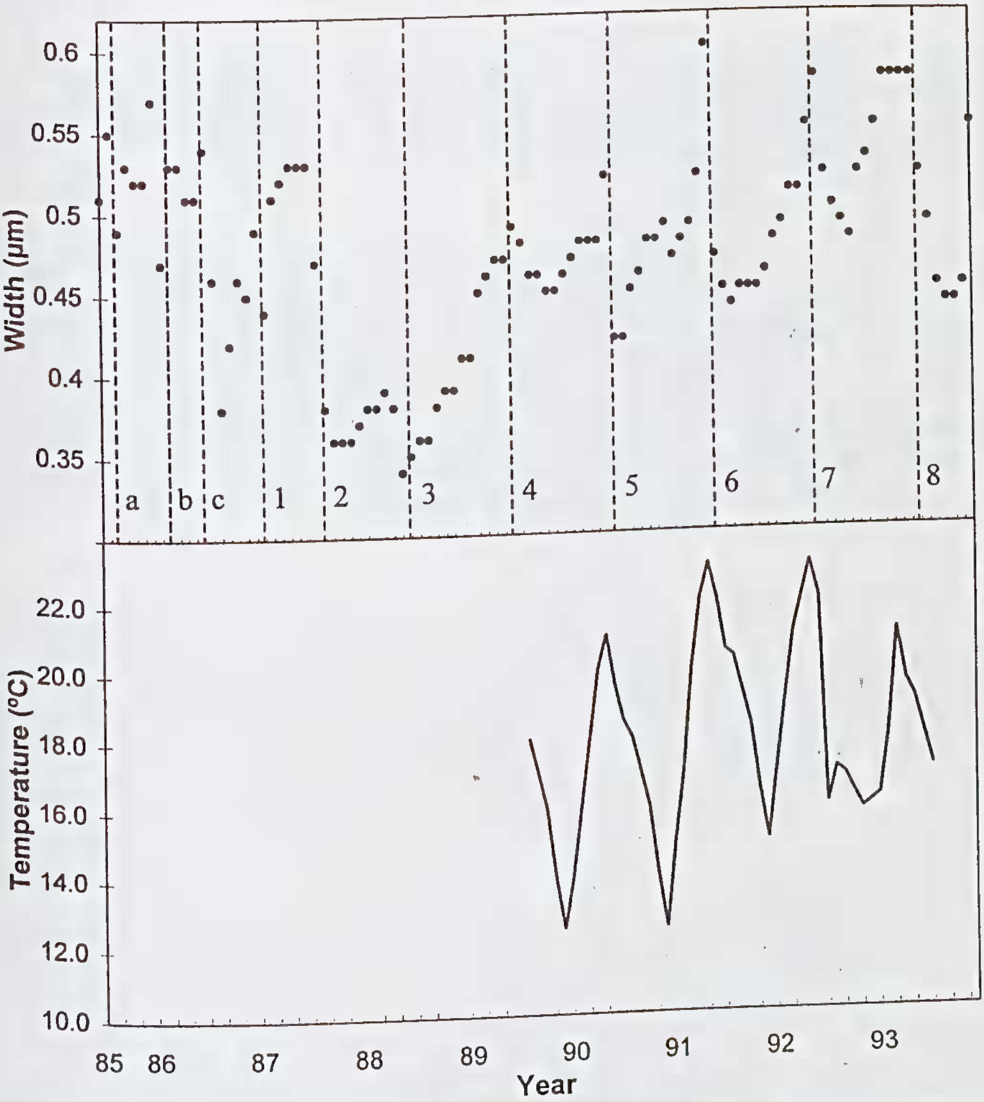

Figure 3. (upper) – plot of change in thickness of aragonitic laminae along a transect from the outer to inner shell surface in a vertical section at the spire of the shell of an 8 yr old animal captured in May 1994. Vertical lines show locations of rings, labelled with letters for fine ones, and with numbers for dark ones. The presumptive time of deposition is given on the x-axis on the assumptions that the dark rings after the first two are deposited annually in about October and that nacre deposition is uniform throughout the year. (lower) – sea surface temperatures for the Cedros- La Natividad region from January 1990 to December 1993 derived in part from Fernandez *et al* (1992) and in part from Gonzalez and Shepherd (1996).

Table 1. Parameters of regression equations of Gulland-Holt plots of annual increment (AI) vs mean length (L). The equations are of the form: AI = a – b L

Time after El Niño (years)	N	a (s.e)	b (s.e)	R^2
1	26	46.5(4.2)	0.28(0.02)	0.846
2.5	34	42.9(3.6)	0.28(0.02)	0.933
3.3	97	42.5(3.5)	0.24(0.02)	0.630

across the section is given in Figure 3. After the deposition of a dark ring the thickness of laminae declines abruptly and then increases again prior to deposition of the next ring. Lamina thickness was significantly correlated with sea temperature ($r=0.39$; $P<0.01$), and even reflected the higher temperatures during the El Niño of 1991–2 (Fig. 3).

Deposition of rings

We have no data on the timing of ring deposition other than in October and November. Nearly all shells taken during these months showed a new ring or part of one in the centre of the horizontal section, indicating very recent deposition. So we provisionally conclude that the dark rings are deposited in about October at about the time of maximum sea temperatures and also spawning.

A plot of the number of rings vs age of the shell for the shells grown in culture (Fig. 4) shows that three fine rings (a–c) are laid down in the first year, the first dark ring at about 20 months, the second soon after two years of age, and the third and fourth at three and four years respectively. A regression of the number of dark rings vs age gave a slope of 1.18 which did not differ significantly from unity ($t_{17}=1.96$; ns). The dark rings are thicker than the fine rings and are pigmented dark brown or black although paler shades also occur.

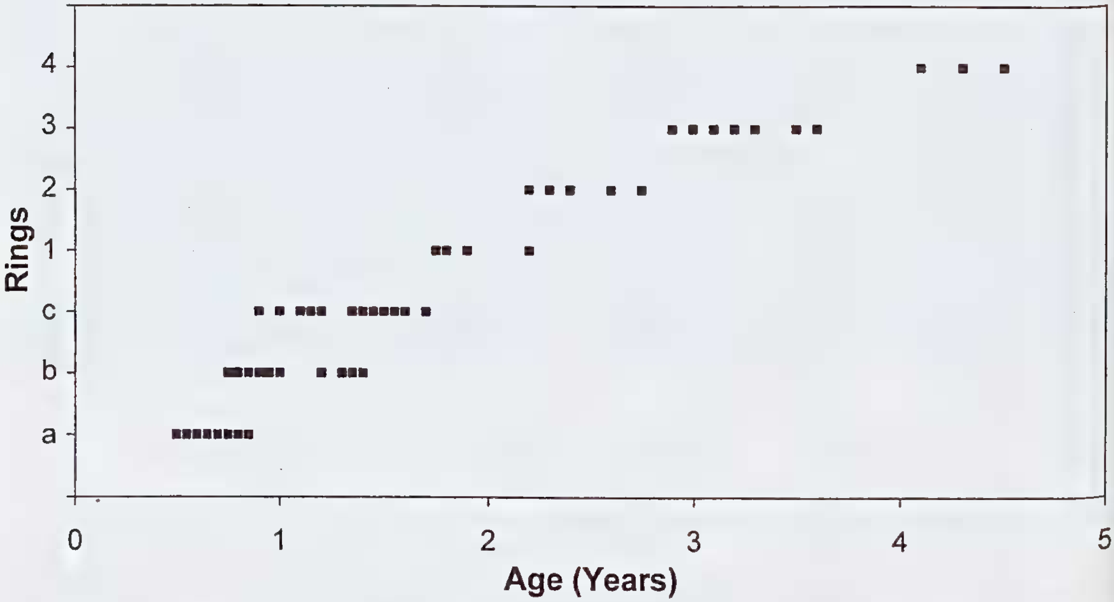

Figure 4. Plot of the number of rings vs estimated age of shells of *H. corrugata* reared in culture. a–c are fine rings and 1–4 are dark rings.

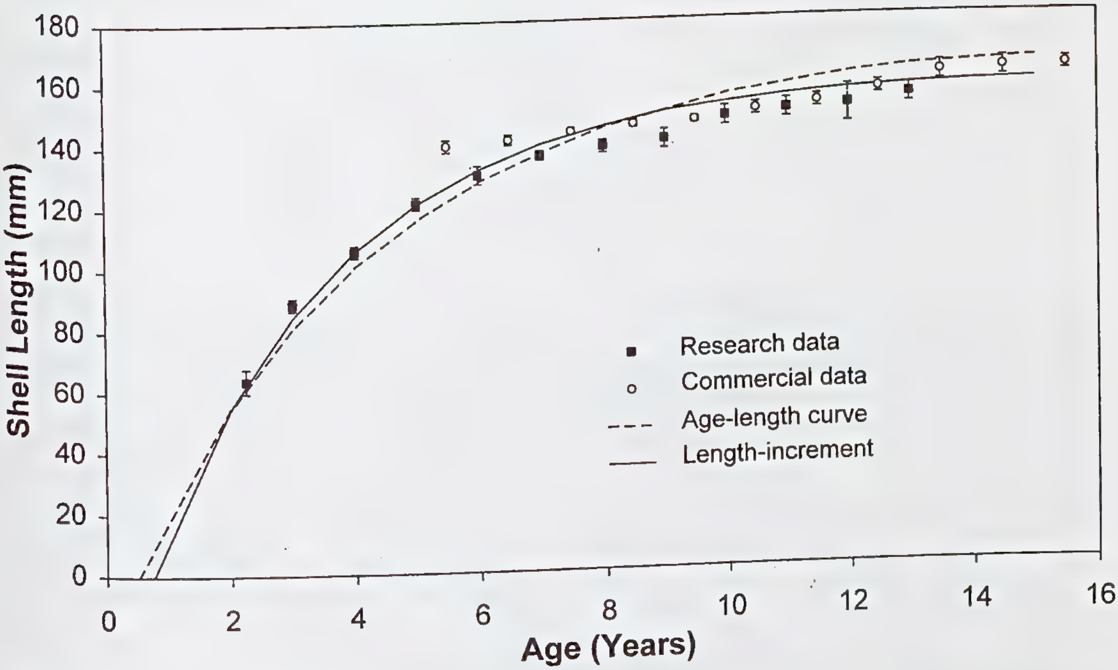

Figure 5. Plot of mean shell length vs putative age in years for data from research shell collections and from the commercial catch. Vertical bars are standard errors. The growth curve fitted to age-length data is shown with a continuous line, and that fitted to length-increment data is shown with a dashed line.

On the assumption that the same timing of deposition occurs in nature i.e. that from age 3 years the number of dark rings gives the number of whole years of age, we plotted mean shell length vs putative age (= number of dark rings) for (a) research shell collections, and (b) commercially caught shells (Fig. 5). Note that the mean length at age is initially higher for the commercial data than for the research data. The legal size at capture is 135 mm SL and the larger size of newly recruited shells presumably reflects the selection by divers of faster growing abalone. The parameters of the von Bertalanffy growth curve fitted to research and commercial data combined are given in Table 2, and plotted in Fig. 5.

Growth check analyses

Examination of 134 shells taken at La Natividad from 1989–1991 showed no prominent growth checks, whereas 50–65% of shells in samples taken from 1992–1995 showed a prominent growth check (Fig. 1a). However, shells with a presumed birth date in 1990 or later showed no growth check. So we concluded that the growth checks were laid down during the El Niño and hypothesised that they were deposited on 1 November 1991 as occurred in *H. fulgens* (Shepherd and Turrubiates 1997) soon after the time of maximum sea temperature. Hence the time periods from the date of deposition of the check would be approximately 1, 2.5 and 3.3 years for the shell samples taken in Nov. 1992, May 1994 and March 1995 respectively. We tested this hypothesis by comparing regressions of mean putative annual growth increment vs mean length (Gulland-Holt plots) for the three data sets. Neither the slopes ($t<1.9$; n.s.) nor the intercepts ($t<1.1$; n.s.) of the regressions differed significantly from each other (Table 1) so we accepted the hypothesis that the time periods were as postulated i.e. that the starting points, the time of deposition of the growth checks, were coincident on about 1 November 1991. Von Bertalanffy growth parameters calculated on the

Table 2. Parameters of von Bertalanffy growth equations for length-increment (LI) data and age-length (AL) data

Data	N	t_o	K yr^{1} (s.e) (mm)	L_∞(s.e.) (mm)	R^2
LI	157	0.46	0.254(0.014)	169.7(1.2)	0.73
AL	13	0.74	0.334(0.031)	159.6(14.0)	0.91

combined length-increment data are given in Table 2. Assuming that L=55 mm at age two, the growth curves of the two equations in Table 2 are presented in Figure 5 for comparison. Their trajectories are very similar and although they cannot be compared by formal statistics, their consistency supports the hypothesis that ring deposition is annual.

Next we plotted the observed number of rings vs mean estimated age in shells with growth checks (Fig. 6). Note that the standard errors of the mean ages increase with age after about 8 years. This is because estimates of age from length, while reasonable during the early nearly linear phase of growth, become increasingly noisy as the growth rate slows. The slope of the regression fitted to the mean estimated ages did not differ significantly from unity (t=1.6; n.s.); but if the means were weighted by the reciprocal of their standard errors in the regression, thereby reducing the weight of older ages, the slope of the regression is 1.11 which is significantly greater than unity (t=2.4; $P<0.05$). The results confirm that to age 12 one dark ring per year is laid down, with a possible accumulated bias of about one extra ring by about age 12 years.

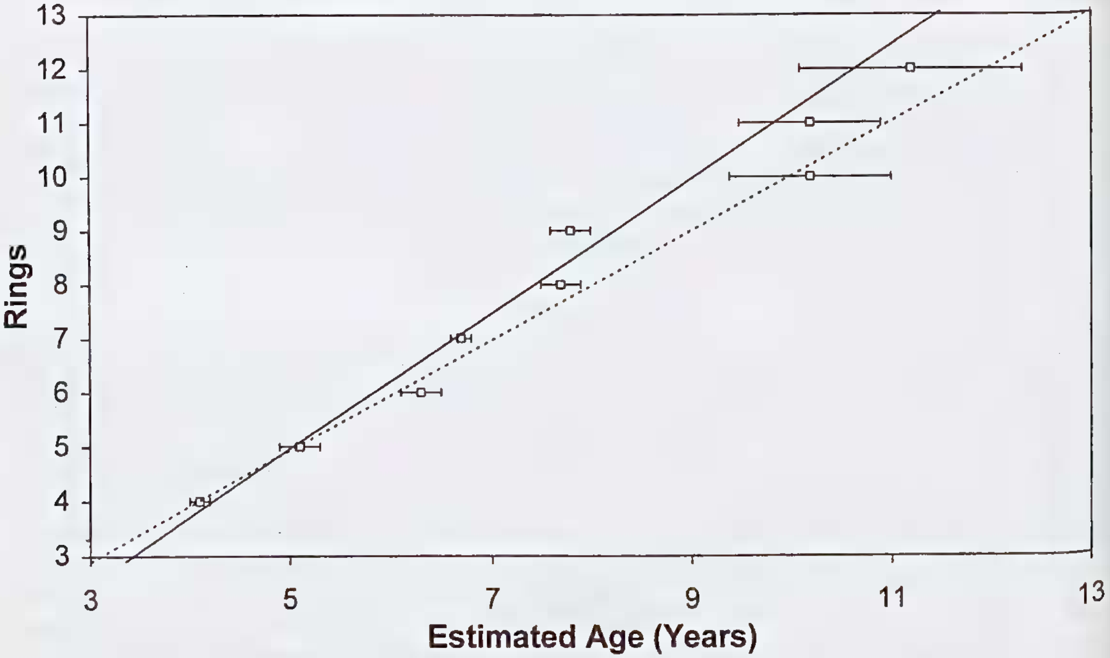

Figure 6. Plot of number of dark rings vs estimated age of shells with El Niño growth checks from the commercial catch taken in 1995. Horizontal bars are standard errors.

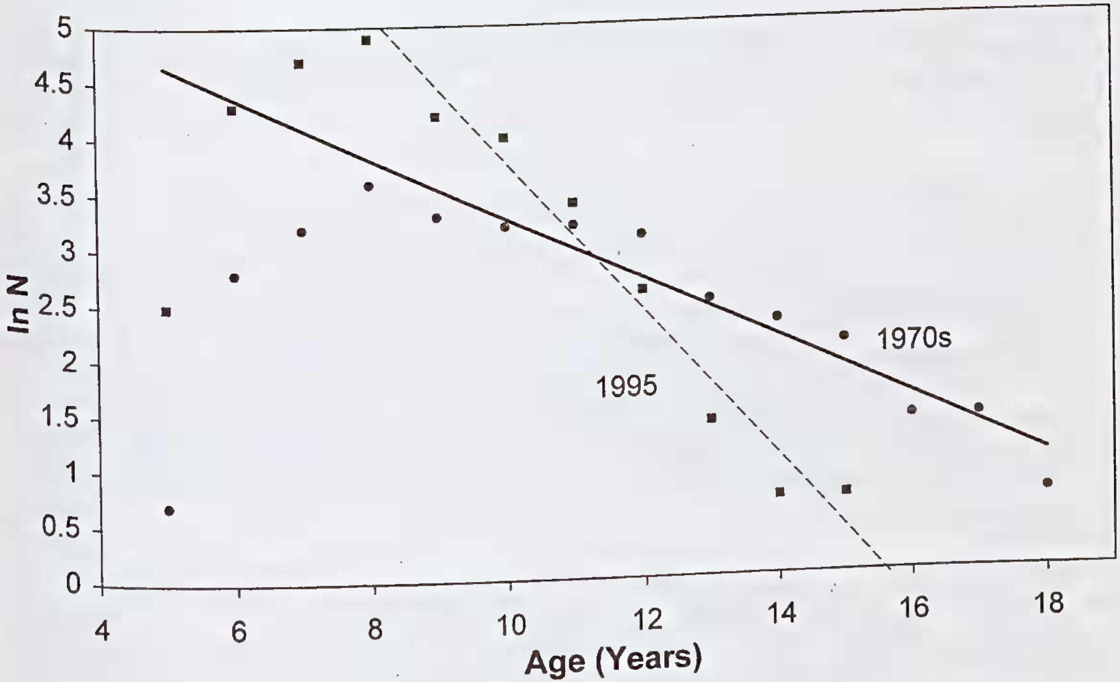

Figure 7. Catch- curve analyses for commercial catch data obtained in the late 1970s and in March 1995. Numbers in the 5–7 year age classes are not fully recruited to the fishery and are excluded from the regressions.

Total mortality rate (Z)

Plots of the natural logarithm of numbers in each age class vs age for the La Natividad samples (Fig. 7) gave estimates of Z of 0.28 (s.e. 0.02) for the late 1970s and 0.66 (s.e. 0.05) for 1995.

Discussion

Shell Microstructure

The microstructure of the rings and the pattern of changes in thickness of aragonitic laminae in *H. corrugata* differ from other species examined (Erasmus *et al.* 1994; Shepherd *et al.* 1995). The prismatic elements of the rings are much coarser and blocklike and the extra granular layer juxtaposed to the prisms has not been seen in other species. The latter is also strongly pigmented suggesting that it is rich in organic matter (conchiolin).

The cyclic change in thickness of laminae in *H. corrugata* is similar to that observed for *H. fulgens* (Shepherd *et al.* 1995a). In both species there is an abrupt decline in thickness after deposition of the dark ring which in both species appears to be laid down annually in October (see Shepherd and Turrubiates, 1997 for the most recent evidence on *H. fulgens*). In *H. corrugata* the correlation of laminar thickness with sea temperature suggests that the cyclic pattern is temperature-dependent as is known for bivalves (Lutz and Rhoads 1980). An alternative hypothesis is that the pattern is associated with maturation and spawning when the dark ring is laid down and laminar thickness declines abruptly. In support of this hypothesis note that the pattern in the first three years of life (following deposition of rings a–c, 1 and 3 in Fig. 3) before the age of sexual maturity differs from that shown later. There are numerous published examples (reviewed by Day and Fleming 1992) where conspicuous interruptions to growth are associated with spawning or temperature extremes (and see also Shepherd *et al.* 1995b). Whatever the cause(s) of the cyclic pattern of changes in laminar thickness, they appear to provide internal evidence of the annual periodicity of ring deposition.

Growth checks

The occurrence of growth checks in abalone shells during strong El Niño events is considered by Shepherd and Turrubiates (1997). These checks are more pronounced in *H. corrugata* than in *H. fulgens*. Given the preference of *H. corrugata* for deeper and cooler water (Leighton 1974; Lindberg 1992; Guzmán del Próo 1992) we postulate that elevated temperatures during El Niños create relatively greater stress on this species than on *H. fulgens* and cause the more prominent check. However we cannot exclude a role of dietary change accompanying the disappearance of *Macrocystis* forests during strong El Niños. In this connection it is worth noting that the El Niño does not appear to have caused more than a brief interruption to growth since there was no significant difference in growth rates for the overlapping periods of 1, 2.5 and 3.3 years after that event (Table 1).

Ring counts

The rings in the spire of *H. corrugata* are very clear and give consistent counts. Horizontal sections are easier to prepare and read than vertical sections, although the latter show more clearly the occasional division of dark rings into two at the spire to produce a false ring (Shepherd *et al.* 1995a). However, with care false rings can be recognised in horizontal sections, too. The fine rings are readily distinguishable from dark rings because they are narrower and lack pigment. In any case they are progressively lost in older shells. But sometimes the first presumptive dark ring is unpigmented and could be mistaken for a fine ring especially in older shells. For this reason careful comparison of the two types of ring is important to avoid misclassification. If there are four (or more) initial fine rings present it is very likely that the excess over three are dark rings lacking pigmentation.

In other species of abalone extra "brown" rings may be deposited in response to parasitic attack (Shepherd and Huchette 1997, Shepherd and Triantafillos 1997)). In a few shells of *H. corrugata* we have seen "brown" rings which are wider and may be coloured more intensely than dark rings. Future studies need to address the question whether parasites can cause the deposition of extra rings in Mexican species as well.

Growth and mortality rates

The estimates of growth and mortality rates presented here are probably the most realistic values yet published for *H. corrugata* in Baja California. Earlier figures given for the region (reviewed by Guzmán del Próo 1992) ($K = 0.19–0.37$; $L_\infty = 159–198$ mm; $Z = 0.5–1.1$) have a wider range than ours, but cannot be critically evaluated because they are based on interpretation of modes of unpublished length-frequency data. Our low value for Z from the late 1970s is especially interesting in the light of the subsequent decline of this species in the 1980s. Thus, aging abalone shells provides the first opportunity in Mexico to obtain accurate parameter estimates for *H. corrugata* for use in age-structured models of the still valuable fishery.

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